SYMPOSIUM ON MICROBIOLOGY OF THE RUMEN[1]

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The importance of microorganisms to the ruminant has interested an increasing number of nutritionists, particularly those in departments of animal science. In view of this interest of agricultural workers, it is surprising to find relatively few rumen microbiologists. Formal discussions on this subject have been held in England and France, but the Rumen Microbiology Symposium at the Fifty-fifth General Meeting of the Society of American Bacteriologists was the first in the United States. It was arranged to present and compare different points of view rather than to summarize current knowledge. A more extensive meeting, including participation by microbiologists from many countries, would be necessary for an adequate treatment of all phases of the subject.

The methods discussed fell into two categories, analyses of the activities of individual types, and study of the integrated activities of part or all of the rumen biota. The analytical approach assumes that for a complete understanding of the rumen fermentation the individual components must be isolated and studied under controlled and reproducible conditions. This involves classical pure culture techniques as well as analyses of the culture requirements and fermentation characteristics of each species. Not only physiological and biochemical problems are encountered, but also knotty questions in morphology, taxonomy, and ecology, each of which may require extended study. If appropriate culture conditions are employed, much of the knowledge about each pure culture probably applies to that organism also in the rumen mixture, but even if abundant information on pure cultures of all rumen species were available, it is doubtful that from the pure culture data alone an accurate picture of the mixed activities could be obtained. For this reason, and because the pure culture approach is time consuming, methods for studying the natural mixture have been developed. The influence of various factors can be tested, the assumption being that their effects *in vitro* and in the rumen will be similar.

The first four papers of the symposium discussed results obtained by the analytical approach. R. E. Hungate reviewed the types of cellulolytic bacteria characteristic of the rumen: *Bacteroides succinogenes*, *Ruminococcus flavefaciens*, colorless cellulolytic cocci, butyric acid-producing nonsporeforming rods, and sporeformers which produce butyric acid. A need for study of fermentation patterns within the genus *Bacteroides* was discussed. The unnamed bacteria need taxonomic study leading to assignment of binomials. The gram reaction and morphology of the colorless cocci would place them in the genus *Veillonella*, but their fermentation pattern differs from the type species in that no propionic acid is formed. M. Rogosa suggested that a new genus should be created for these colorless cocci.

The fermentation products of the cellulolytic bacteria, as found in the pure cultures, include H_2, CO_2, formic, acetic, butyric, lactic, and succinic acids, and ethanol. The acetic and butyric acids and carbon dioxide are final products also in the rumen. Conversion of lower fatty acids into higher ones has been found in pure cultures and may occur also in the rumen. Lactic acid is converted via two fermentations, one a propionic type with also acetic acid and CO_2 formed, and one a butyric type with also acetic acid, formic acid, CO_2, and H_2. Formic acid is decomposed to H_2 and CO_2. Succinic acid is decarboxylated to propionic acid. Hydrogen is used for the reduction of CO_2 to CH_4. Ethanol is not a final product in the rumen; it presumably is formed, but its further conversions have not been determined.

The analytical approach has also been applied to the rumen protozoa, though pure cultures have not been obtained. The role of the protozoa in the rumen was reviewed by J. Gutierrez. Procedures for mechanically separating the protozoa

[1] This symposium consisted of six formal papers and a following discussion. Lack of time prevented the participants from preparing any mutually acceptable formulation of principles of rumen microbiology. This summary by the convenor, R. E. Hungate, is an attempt to indicate the chief accomplishments and viewpoints expressed.

[2] Convenor.

of the genera *Dasytricha* and *Isotricha* were described, and the results of manometric experiments on metabolic products and rates of metabolism by these genera were reported, with estimates of the magnitude of the fermentation by the other protozoa. The activities of the protozoa accounted for about 20 per cent of the total fermentation products.

In addition, nitrogen determinations were run on the rumen holotrichs and from the rate of division the amount of nitrogen supplied the host in the form of protozoa was estimated to be 10 to 20 per cent of the total nitrogen requirements. Since the numbers and kinds of protozoa vary widely with time, their importance in the rumen is also variable. The work with the protozoa is of particular interest because it represents an attempt at both analysis and integration, with the analytical data used to estimate activity in the rumen.

The analytical approach was followed also in the papers on the growth requirements of the cellulolytic bacteria. M. P. Bryant described experiments leading to the discovery that certain volatile acids were essential nutrients for *Bacteroides succinogenes*. Common complex organic media did not support growth, but mixtures of the common B-vitamins plus purines and pyrimidines were adequate, if the volatile acids from rumen fluid were included in a concentration equivalent to 20 per cent rumen fluid. Further analysis showed that a branched-chain and a straight-chain fatty acid were necessary. The branched chain-acid requirement was met by isobutyric, isovaleric, and α-methyl-*n*-butyric acids; the straight chain-acid requirement by *n*-valeric, *n*-caproic, *n*-heptanoic, and *n*-caprylic acids, with palmitic and stearic acids less effective.

In the rumen the acid requirements are probably supplied by isobutyric, isovaleric, D-α-methyl-*n*-butyric acid, *n*-valeric, and *n*-caproic acids, all of which are present in adequate concentration. These acids are formed in the rumen through fermentation by accompanying bacteria; *Bacteroides succinogenes* is dependent on their activity. The early observation that proteins in the feed greatly stimulated cellulose decomposition may be explained by a production of the essential volatile acids from amino acids.

The analytical approach was represented also in the study of the growth requirements of a cellulolytic coccus by D. W. Fletcher. Like *Bacteroides succinogenes*, this organism cannot grow on the usual complex organic media and requires rumen fluid, but the essential factor is different. Volatile acids from the rumen cannot substitute for rumen liquid. A microbiological assay method was developed and used to follow the concentration of the unknown during attempts to concentrate and purify. Identified characteristics were: organic; stable to desiccation, moderate alkalinity and acidity, and heat (121 C); water-soluble; non-volatile; non-protein; dialyzable; and stable to mild oxidation.

The mixed culture approach was represented in the last two papers of the symposium. C. N. Huhtanen discussed briefly the direct count and the culture count as measures of the total rumen population and pointed out inadequacies of each for quantitative estimates of microbial activity. The direct count was reported as one hundred billion cells per ml and the culture count as ranging between one and ten billion per ml.

The paper dealt chiefly with *in vitro* incubation of rumen liquid as a method for studying the over-all importance and activity of the mixed rumen organisms. Time did not permit a summary of results of the extensive experiments of the numerous investigators following this approach, but the advantages and disadvantages and some of the data obtained by the speaker were presented. Data pertinent to feeding trials could be obtained quickly and at low cost. Activities *in vitro* resembled those in the rumen if incubation was limited to 16 to 24 hours, but longer periods caused death of protozoa and an increase in aerobes and putrefactive bacteria. During short incubation periods non-protein nitrogen loss was not equivalent to protein synthesis. Inhibition by glucose of the digestion of alfalfa hay was removed if urea was also added. The percentage of the alfalfa digested was independent of the amount supplied, *i.e.*, a constant proportion was digestible.

It was emphasized that results obtained from *in vitro* incubation are not necessarily directly applicable to the rumen, but by duplicating insofar as possible the conditions of the rumen the results become increasingly reliable.

The last paper, by R. N. Doetsch, discussed the value of manometric studies, using washed suspensions of rumen bacteria. The rumen contents were considered a tissue that was relatively

uniform in composition and performed certain functions at a fairly constant rate which can be measured manometrically. It is still necessary to check the results against the intact rumen, but a quick estimate of the kinds of activities represented and their relative magnitude can be obtained.

Certain factors in the washed suspension technique differ from those of the rumen. Among the important ones are: exclusion of the protozoa, absence of the normal fibrous substrates and the bacteria which may cling to them. It is difficult to relate the reaction rates of the washed suspensions to the rates in the rumen, and estimates of amounts of materials produced in the rumen itself are not obtained by this method. On the other hand, fewer variables are involved, and the nature of the substrates and the amount of growth can be controlled. For comparative studies of the integrated activities of the rumen bacteria and for identification of particular reactions, the washed suspension technique is a valuable tool.

Although, throughout the symposium, the possible practical applications of rumen microbiology were mentioned, much of the emphasis was on purely biological aspects. The rumen is an intriguing ecological niche, of inherent interest because of the anaerobiosis, the numerous kinds of microorganisms, and the rapid conversions of organic materials. In many respects the rumen resembles soil. In both habitats a multiplicity of kinds of microorganisms converts a multitude of substrates into materials of great agricultural importance. In the rumen, greater constancy of temperature, water, substrate, and removal of metabolic products, results in a more stable population than that of soil. The reactions are more easily measured because of their greater magnitude. The rumen is thus a habitat particularly favorable for microbiological and biochemical analysis. Knowledge of the actions and interactions of the rumen organisms may provide clues to difficult analyzable interrelationships in soil and other natural habitats. In addition, animal feeding practices may be improved.